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Vimentin gene: expression in human lymphocytes and in Burkitt's lymphoma cells

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We have isolated a human genomic clone for the intermediate filament subunit vimentin with a DNA probe encoding chicken vimentin. We show that the gene for this protein exists as a single copy in the haploid human genome and is transcribed into one mature RNA species of 2 kb. *In vitro* translation of poly(A)$^+$ mRNA in a rabbit reticulocyte cell-free system showed that vimentin is a major product of RNA from normal lymphocytes but not of RNA extracted from Burkitt cells. 2-kb vimentin mRNA can be detected with a DNA probe in normal lymphocytes and in fibroblasts, but not in cell lines derived from Burkitt's lymphoma (JI, JBL2, BJAB, DAUDI). The abundance of vimentin mRNA is correlated with the quantity of vimentin present in the cells, suggesting that the level of expression is regulated by the abundance of mRNA. The half-lives of vimentin mRNA were found identical in both fibroblasts and lymphocytes and belong to the class of stable mRNA.

Key words: intermediate filaments/vimentin translation/genomic clone/mRNA/chromosomal translocation

Introduction

Vimentin is the major intermediate filament protein in the cytoplasm of mesenchymal derivatives (for review, see Traub, 1985). In lymphoid cells, level of expression and cellular organization are variable within the different lineages. Whereas human T lymphocytes retain vimentin expression at all stages of maturation, B lymphocytes reduce vimentin synthesis when they mature into plasma cells (Dellagi *et al.*, 1983). Tumor cells derived from B lymphocytes such as Burkitt's lymphoma cells, do not express vimentin, demonstrating that vimentin is not required for rapid cell proliferation (Dellagi *et al.*, 1984; Mortazani-Milani and Holborow, 1983). When cultured as permanent cell lines, Burkitt's lymphoma cells do not acquire vimentin expression in contrast with all the mammalian cells grown *in vitro* (Francke *et al.*, 1979). Similarly, no vimentin is detected in the mouse equivalent of human Burkitt's lymphoma cells such as myeloma cells. Consistent with the absence of vimentin from mouse myeloma and plasmocytoma, poly(A)$^+$ RNA of both cell lines does not code for vimentin in a cell-free system (McTavish *et al.*, 1983). This result suggests that levels of vimentin are regulated at the transcriptional level rather than, for example, by the action of a specific Ca^{2+}-activated proteinase (McTavish *et al.*, 1983). Since direct quantitation of vimentin mRNA with Northern methods has not yet been performed, questions arise about the abundance and stability of vimentin transcripts. We have therefore compared levels of vimentin RNA in lymphocytes and Burkitt's lymphoma cells. Our analyses included human fibroblasts and B and T lymphocytes as well as vimentin-negative Burkitt cell lines (DAUDI, RAJI, JBL2, BJAB, JI) and a low vimentin producer BL13. We measured the abundance and size of vimentin transcripts by hybridization with a DNA probe encoding chicken vimentin (Zehner and Paterson, 1983a). To investigate the possibility of a post-transcriptional control we examined the stability of vimentin mRNA in actinomycin and cycloheximide-treated cells. As an internal control, actin was monitored in parallel. The amount of

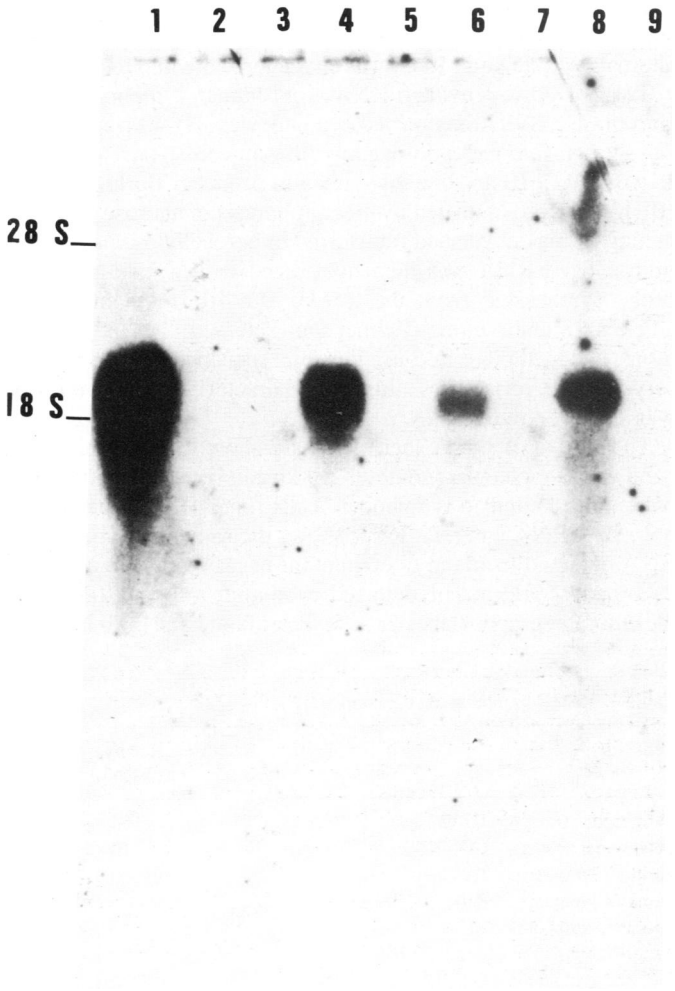

Fig. 1. Detection of vimentin RNA transcripts in different cell types. RNA from SV40-transformed human fibroblast **(lanes 1, 8)**, EBV-transformed human B lymphocytes **(lane 4)** or Burkitt's lymphoma cells **(lanes 2:** BJAB: **3:** JI; **5:** JBL2; **6:** BL13; **7,9:** DAUDI) were fractionated on a 1.5% agarose gel transferred to nitrocellulose filters and hybridized with the probe. Markers run on the gel were 28S RNA (5000 bases) and 18S RNA (1940 bases). **Lanes 1–9** are loaded with 20 μg of total RNA except **lanes 8** and **9** with 2 μg. 5×10^6 c.p.m./ml of ^{32}P-labelled chicken insert were used. In **lane 6** a faint band of 2 kb length is detected corresponding to Burkitt cell BL13 in which 20% of cells are positive for vimentin. Blots were overexposed.

vimentin mRNA was compared with vimentin polypeptide synthesized *in vitro* in a cell-free system and *in vivo* after radioactive labelling. Actin and tubulin polypeptides were chosen for relative quantification of protein synthesis. To complete our studies, a vimentin recombinant clone was isolated from a human genomic library in order to characterize the human vimentin gene.

Results

Detection of vimentin RNA transcripts in different cell types

We have previously shown that vimentin extracted from normal lymphocytes migrate in 2D gels to its expected position and that vimentin accounts for ~0.6% of the cytoskeletal components. In extracts from Burkitt cells, vimentin was undetectable on 2D gels. The lack of vimentin in Burkitt cell extracts was in agreement with the absence of intermediate filaments detectable with immunofluorescence.

From these results, it seems possible that the absence of vimentin from Burkitt cells was due to a lack of vimentin mRNA. However, it was possible that vimentin mRNA was transcribed but stored in an untranslatable form. In order to decide between these alternatives, vimentin RNA was characterized with DNA probes.

Total or polyadenylated RNA from human lymphocytes and fibroblasts, EBV-transformed lymphocytes, SV40-transformed fibroblasts, five vimentin-negative Burkitt cells (RAJI, DAUDI, BJAB, JBL2, JI) and one low vimentin-producer Burkitt cell line (BL13) were fractionated by electrophoresis in agarose gels containing formaldehyde and transferred to nitrocellulose paper. The immobilized RNA was then hybridized with a nick-translated probe synthesized from the 2.9-kb *Hin*dIII fragment from a chicken vimentin clone (Zehner and Paterson, 1983a; see Materials and methods). Because vimentin sequences are highly conserved in all vertebrates, this well characterized chicken probe was used for the studies.

The results of such experiments are shown in Figure 1. One band with a molecular length of 2.0 kb can be identified in fibroblast (lane 1) and in lymphocyte cells (lane 4). Comparison of poly(A)$^+$ RNA and total RNA shows the same 2-kb species. In RNA obtained from the five vimentin-negative Burkitt cells, the 2-kb band could not be detected even after a long exposure of the autoradiographs (lanes 2, 3, 5, 7). A faint band 2 kb in length is detected for Burkitt cells BL13 which is known to produce a low amount of vimentin (lane 6). Prolonged exposure of the blot does not reveal the presence of other RNA species in normal or in Burkitt cells. In order to quantify the vimentin mRNA, the 2-kb bands from the seven lanes were excised from nitrocellulose paper and the radioactivity measured in a scintillation liquid counter. Relative levels are respectively 100, 10 and 3, for fibroblasts, lymphocytes and BL13; no radioactivity corresponding to mRNA was detected for other Burkitt cell lines (Table I).

As a control, similar blots were probed with a nick-translated insert synthesized from β-actin clone (Alonso *et al.*, 1986). For fibroblasts, lymphocytes and Burkitt cells, the 2-kb RNA coding for β-actin was revealed. The amounts of actin mRNA were measured by excising the spots from nitrocellulose paper and counted as for vimentin RNA (see Table I).

In vitro translation of mRNA transcripts from lymphocytes and lymphoma Burkitt cell lines

To determine whether vimentin synthesis was also controlled at the translational level, poly(A)$^+$ isolated from fibroblast, lymphocytes and Burkitt cells was translated in a mRNA-dependent reticulocyte lysate. The translation products were then analysed by two-dimensional polyacrylamide gel electrophoresis. A spot is seen corresponding to a protein with a mol. wt of 56 kd and pHi 5. This translation product was identical to vimentin with regard to its mol. wt and pHi. By excising this spot from the dried gel and counting it in scintillation liquid, the protein was estimated to account for a maximum of 3% for fibroblasts and 0.8% for lymphocytes of the translated polypeptides (Figure 2).

Table I. Relative amounts of vimentin RNA

Cells		Relative amount of RNA
SV fibroblast	JDA2	100
EBV lymphocytes	IARC 272	10
Burkitt's lymphoma	JI	< 0.1
Burkitt's lymphoma	BJAB	< 0.1
Burkitt's lymphoma	BL13	3
Burkitt's lymphoma	JBL2	< 0.1
Burkitt's lymphoma	DAUDI	< 0.1

The relative amount of vimentin RNA was measured after excision of the different spots corresponding to lanes 1–7 (see Figure 1) and counting in a scintillation liquid spectrophotometer. The total values counted were from 1000 to 100 000 c.p.m. Each lane was loaded with 20 μg of total RNA extracted from various cells. RNAs were then fractionated on a 1.5% agarose gel, transferred to nitrocellulose filters and hybridized with the vimentin probe. As a control of the RNA preparation, similar blots were probed with a nick-translated insert synthesized from a β-actin clone. The radioactivity was measured as for vimentin RNA. The respective amounts for the seven lines were (1) 1400, (2) 1700, (3) 900, (4) 1300, (5) 700, (6) 700, (7) 800 c.p.m.

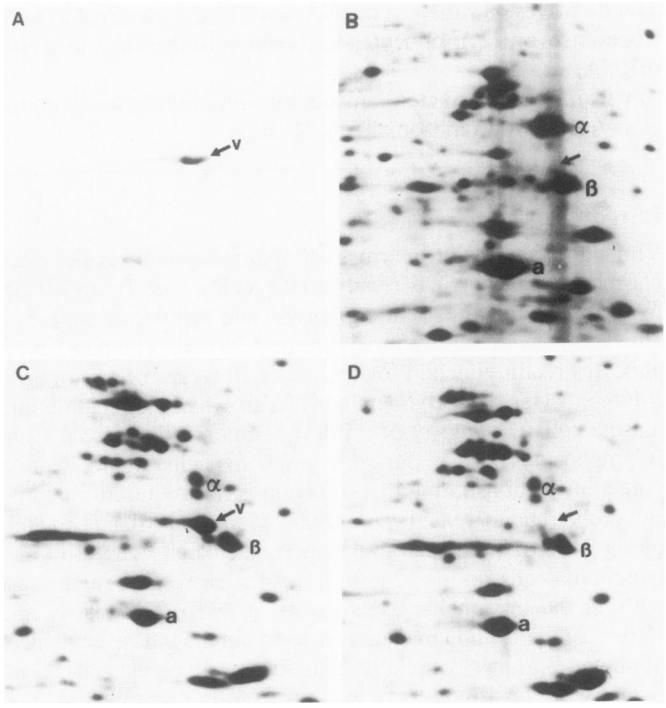

Fig. 2. Autoradiograph of 2D electrophoresis from polypeptides labelled *in vivo* with [^{35}S]methionine and extracted from Burkitt **(B)** and of translation products coded for by poly(A)-rich RNA lymphocytes **(C)** of Burkitt cells **(D)**. Arrows point to the positions of the vimentin polypeptides.
(A) Autoradiograph of 2D electrophoresis gel of translation products obtained following hybridization selection of the mRNA. 20 μg of the *Eco*RI–*Bam*HI fragment from HuVim5 was spotted onto nitrocellulose; 100 μg of total RNA extracted from human fibroblasts were incubated in the appropriate buffer. Bound RNA was eluted then precipitated. Translations were carried out in a reticulocyte lysate system.

Poly(A)$^+$ RNA was isolated from Burkitt cell lines DAUDI and JI, and translated under the same conditions as the lymphocytes. Analyses of translation products by 2D polyacrylamide gel electrophoresis show that while a large number of proteins were synthesized, vimentin was undetectable even after a long exposure. Determination of the relative amounts of other cytoskeletal proteins in the translation products encoded by poly(A)$^+$-rich RNA from lymphocytes and from Burkitt cell line DAUDI, by counting of excised spots, gave identical values for the amount of actin and tubulin; 3% and 1.5%, respectively. Because it was possible that some mRNA was not polyadenylated, a fraction of RNA lacking poly(A) was also used to direct protein synthesis in the reticulocyte lysate. No vimentin was detected in the translation products coded by RNA lacking poly(A) (data not shown).

Since a Ca^{2+}-activated neutral thiol proteinase which has a high affinity for vimentin has been found (McTavish *et al.*, 1983), the question arises whether the amounts of vimentin detectable *in situ* are modulated by proteinase activity *in vivo* or are due to differences in the efficiency of translation of mRNA coding for vimentin. Cytoskeleton from 10^6 cells labelled with [^{35}S]methionine was extracted and analysed by 2D gel electrophoresis. The radioactivity associated with vimentin, actin and tubulin spots was determined. Actin and tubulin spots give identical values for lymphocytes or Burkitt cells, but in Burkitt cells no radioactive spot was seen at the vimentin position (Figure 2). The results of relative *in vivo* and *in vitro* syntheses of cytoskeletal proteins are shown in Table II.

Table II. Relative *in vivo* and *in vitro* syntheses of cytoskeletal proteins

Polypeptides	Radioactivity			
	In vivo		*In vitro*	
	Lymphocytes	Burkitt cells	Lymphocytes	Burkitt cells
Vimentin	0.6 ± 0.1	0	0.8 ± 0.10	0
Actin	3.4 ± 0.2	3.4 ± 0.2	3 ± 0.15	3 ± 0.15
α- and β-tubulin	1.6 ± 0.1	1.6 ± 0.1	1.4 ± 0.10	1.5 ± 0.1

Areas from the gel (see Figure 2) corresponding to actin, α- and β-tubulin and vimentin were excised, dissolved in tissue solubilizer and counted. Results are an average of measures obtained from four gels. Usually each gel was loaded with 1 × 10^6 c.p.m.

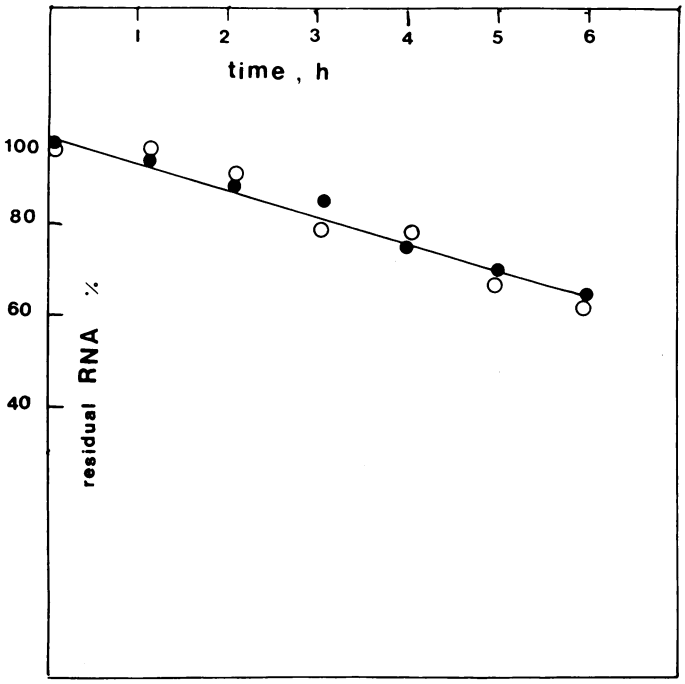

Fig. 3. Stability of vimentin mRNA from cells treated with actinomycin D. Cells were treated with 1, 5 or 10 μg of actinomycin D for various times. For each time point 10 μg of RNA were analysed. ○ lymphocytes, ● fibroblasts.

Stability of vimentin mRNA

Measurements of vimentin messenger lifetimes were made with fibroblasts, lymphocytes and Burkitt cells. RNA was extracted from cells, before and at various times after treatment with different doses of actinomycin D (1, 5 and 10 μg/ml) to block essentially all transcriptional activity. Each sample was then electrophoresed under denaturing conditions, transferred to nitrocellulose and analysed for its content of vimentin mRNA. Autoradiography of RNA hybridizations from fibroblasts and lymphocytes before and after actinomycin chase revealed the same 2-kb vimentin RNA species. A detailed kinetic analysis over 6 h followed by quantitative densitometry of the autoradiograph allowed the determination of a half-life >6 h for vimentin RNA. These experiments show that transcripts from both fibroblasts and lymphocytes appear to be comparably stable (Figure 3).

To determine if the absence of vimentin mRNA could be due to a labile protein that either acts as a repressor or in some way interferes with mRNA stability, Burkitt cells were incubated with cycloheximide (20 μg/ml). RNA extracted from lymphocytes and Burkitt cells before and after 3 h exposure to cycloheximide was analysed for vimentin mRNA. For both fibroblasts and lymphocytes, no obvious modifications were found. For Burkitt cells (DAUDI) no band appeared, even after long exposure of the blots. For the low vimentin producer BL13 line, the amount of RNA hybridizing with the vimentin probe was identical before or after 3 h treatment with cycloheximide (20 μg/ml).

Isolation and characterization of human vimentin gene

To allow a more detailed study of the vimentin gene, recombinant DNA clones containing vimentin gene sequences were isolated from a human DNA (EMBL4) library (for details, see Materials and methods). The library was screened with the ^{32}P-labelled fragment of the chicken genomic clone used above (Zehner and Paterson, 1983a). Four positive hybridizing clones were isolated and analysed. Only one, called λV8, 15 kb in length, contained sequences coding for the vimentin gene. Partial sequencing and hybridization to chicken vimentin probe showed that one half of the clone (8 kb) includes most of the vimentin gene and the polyadenylation signal; the other half (7 kb) contains 3′-flanking DNA. Plasmids containing genomic vimentin sequences were constructed by ligation of *Eco*RI−*Bam*HI and *Hin*dIII-digested fragments of the genomic λV8 clone which had been digested with the appropriate enzymes to generate complementary ends. The 5-kb, *Eco*RI−*Bam*HI fragment, called HuVim5, was used to characterize the clone as a part of the vimentin gene in the following experiments. (i) The ^{32}P-labelled HuVim5 probe, hybridized to RNA from human fibroblasts and lymphocytes, revealed one band of 2 kb. This band was equivalent in size to that revealed by hybridization with the chicken vimentin clone. No band was revealed in Northern blots of RNA from Burkitt cells (Figure 4). (ii) Analysis by hybrid-selected translation shows that RNA from fibroblasts which bound specifically to HuVim5 codes for a protein with a mol. wt of 56 kd. The identity of this translation product as vimentin was confirmed by 2D gel analyses (Figure 2). In Southern blot exper-

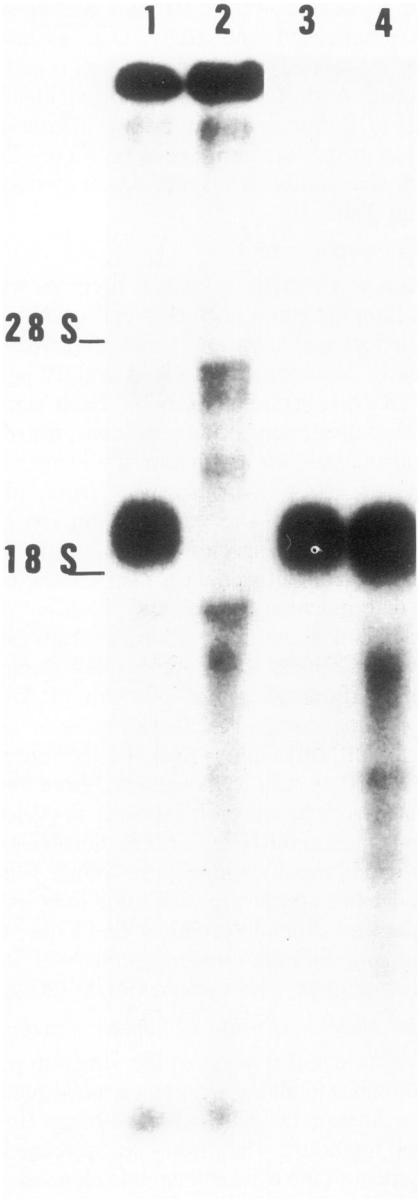

Fig. 4. Detection of vimentin RNA transcripts with a human vimentin clone. The 5-kb *Eco*RI–*Bam*HI fragment (5×10^6 c.p.m./ml) was hybridized to RNA from human fibroblasts **(lane 1)** or Burkitt cells (DAUDI) **(lane 2)**. Note the band at the top of the blot due to repetitive sequences in the human DNA. As a control of the RNA preparation, identical blots were probed with a nick-translated insert synthesized from mouse β-actin cloned (1×10^6 c.p.m./ml) RNA from fibroblasts **(lane 3)** and Burkitt cells (DAUDI) **(lane 4)**.

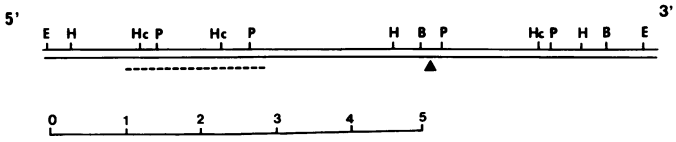

Fig. 5. Map of the human vimentin gene. Restriction enzyme sites were determined by using single and double digests from the λV8 clone and plasmid subclones. Enzymes used: E: *Eco*RI; P: *Pst*I; H: *Hin*dIII; Hc: *Hin*dII. Scale shown is in kb. Arrowheads indicate the 3′ end of the coding region. The 5′ end of the gene is not present in the clone that begins in the intron II. Dots indicate regions which were partially sequenced corresponding to a segment of 1450 nucleotides encoding for amino acids 207–344.

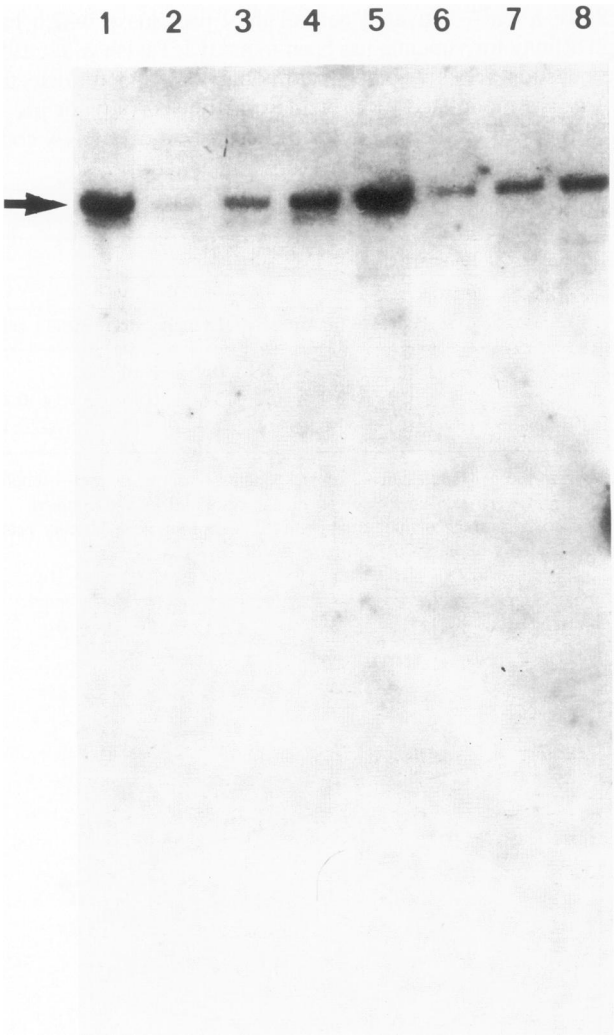

Fig. 6. Detection of vimentin sequences in the human genome with DNA blot hybridization. DNA from lymphocytes and Burkitt's lymphoma cells was digested with *Eco*RI or *Bam*HI, fractionated on 0.6% agarose gels, transferred to nitrocellulose filters and hybridized with ^{32}P-labelled nick-translated vimentin probes from human or chicken. 10 μg of DNA was loaded in all cases except for **lanes 2** and **4** where only 3 μg was loaded. **Lanes 1–4**, *Bam*HI digests; **5–8**, *Eco*RI digests. DNA from B lymphocytes **(lanes 1, 5)**; T lymphocytes KE37 **(lanes 2, 6)**; Burkitt's lymphoma cells JBL2 **(lanes 3, 7)**; BAJI **(lanes 4, 8)**. A very faint additional band appeared at 10.5 kb corresponding to cross-hybridization with desmin intermediate filament, which is known to share homology with vimentin intermediate filament (Geisler and Weber, 1983; Quax *et al.*, 1984, 1985; Lazarides *et al.*, 1984).

iments, this clone shows a smear characteristic of repetitive sequences homologous to those present in human DNA.

Restriction analysis of the human genomic clone λV8, determined by using single and double digests, is represented in Figure 5.

Vimentin-specific sequences in the human genomes

High mol. wt human DNA prepared from B lymphocytes, T lymphocytes and Burkitt's lymphoma cells JBL2 and RAJI, was digested with restriction endonuclease *Eco*RI, *Bam*HI or *Hpa*II. After electrophoresis and transfer to nitrocellulose paper, DNA was hybridized with ^{32}P-labelled probe from the 2.9-kb *Hin*dIII

Table III. Characteristics of human cells

Name	Type	Cytogenetic findings	Vimentin expression
JD A2	SV-transformed fibroblasts	T antigen$^+$	+
CEYSAC	Secondary culture of fibroblasts		+
B lymphocytes	Fresh human blood lymphocytes		+
IARC 272	EBV-transformed B lymphoblasts	EBV$^+$	+
KE 37	T-lymphocytic line	EBV$^-$	+
RAJI	Burkitt line	EBV$^+$, translocation 8,14	−
JI	Burkitt line	EBV$^+$, translocation 2,8	−
JBL2	Burkitt line	EBV$^-$, translocation 2,8	−
BJAB	Burkitt line	EBV$^-$, translocation −	−
DAUDI	Burkitt line	EBV$^+$, translocation 8,14	−
BL13	Burkitt line	EBV$^+$, translocation 8,14	±

See Klein and Lenoir (1982).

fragment of chicken pVim. Figure 6 shows that with these endonucleases only one fragment is detected. In *Eco*RI or *Bam*HI digests the unique band has a length of 11 kb. With *Hpa*II fragments of DNA from lymphocytes or Burkitt cell digests, one unique band of 6.5 kb hybridized with the vimentin probe. Genomic DNA blot analysis demonstrated the existence of a single gene in the human genome confirming the result that Quax *et al.* (1985) reported while this work was in progress.

No differences were detected between normal B, T lymphocytes and Burkitt's lymphoma cells, suggesting that no major rearrangements of the vimentin gene occurred in these tumoral cells.

Discussion

Two main conclusions emerge from our results. First, there is only one species of vimentin RNA of 2 kb length produced in lymphocytes and human fibroblasts whereas as shown by Zehner and Paterson (1983b) for chicken myogenic cells and by Capetanaki *et al.* (1983) for erythroid cells, two vimentin mRNAs of 2 kb and 2.3 kb length are present, which originate from multiple poly(A) addition signals at the 3′ end of the gene. The hamster vimentin gene has never been shown to express more than one RNA (Dodemont *et al.*, 1982) and is similar to the human gene in this respect. By contrast, Burkitt cell lines RAJI, DAUDI, BJAB, JBL2 and JI, which are not able to produce vimentin, possess neither 2-kb RNA nor mRNA species homologous to vimentin probes.

Second, vimentin mRNA belongs to the class of stable mRNAs with a half-life >6 h. Cycloheximide, a protein synthesis inhibitor, appears to be without effect on the stability and the amount of vimentin mRNA, excluding the hypothesis of a control by a labile protein at the post-transcriptional level (Dani *et al.*, 1984). In spite of the fact that the rates of gene transcription have not been measured in isolated nuclei, our results permit us to eliminate the possibility of a change in the rate of mRNA turnover and therefore to conclude that vimentin is regulated in both cell types, mainly at the level of transcription. In all the cells tested, RNA hybridizing with vimentin probe was able to direct the synthesis of vimentin in a cell-free system. Assuming that the efficiency of the *in vitro* translation was similar for the vimentin RNA from various cell types, we found a good relationship between the amount of vimentin RNA detected and vimentin translated *in vitro*. Fibroblasts contain the highest levels of vimentin RNA and the highest amount of vimentin *in situ*. Levels of vimentin mRNA and protein were lower in the low producer Burkitt cells BL13. Although no titration experiments have been carried out, the observation of a single band pattern in genomic blots with three different restriction fragments in the human recombinant clone pHu-Vim5 confirm that the sequence for the vimentin subunit occurs only once in the haploid human genome. It has been reported that vimentin intermediate filament is also encoded by a single gene on human chromosome 10 and preliminary data indicating that vimentin is encoded by a single gene were reported while this work was in progress (Quax *et al.*, 1985). The Burkitt cells studied had a characteristic translocation involving the distal end (band q24) of one of the 8th chromosomes and chromosome 14, 2 or 22 leading to the transposition of the *myc* oncogene (Robertson, 1984). No differences in the restriction sites of the DNA extracted from B, T lymphocytes or Burkitt cells were detected, suggesting that no major rearrangements of the vimentin gene occurred in these tumoral cells.

Materials and methods

Isolation of genomic clones

A human genomic library of *Sau*IIIA random fragments inserted in the *Bam*HI site of the EMBL4 phage was used. 4×10^5 recombinant phage plaques (~2 haploid genomes) were screened with a chicken vimentin probe. The chicken probe was a *Hin*dIII fragment extracted from a chicken vimentin clone isolated by Zehner and Paterson (1983a). The clone λV8 contains an 8-kb *Eco*RI–*Bam*HI insert with only 4 kb of coding sequences and intronic sequences localized in the 3′ part of the gene. A 2.9-kb *Hin*dIII fragment contained in this coding region was used as probe because it was the only *Hin*dIII fragment which recognized a unique fragment in the chicken genome digested by *Hin*dIII. 1 μg of this probe was nick-translated using [^{32}P]dATP and [^{32}P]dCTP (3000 Ci/mmol, Amersham). All the screening was performed with a specific activity of $\sim 5 \times 10^8$ c.p.m./g. The recombinant plates were transferred and hybridized by standard methods (Benton and Davis, 1977) and the filters were washed in 0.1% SDS, 0.5% SSC at 60°C. Six positive clones (1, 16, 18, 23, 25, 43) were isolated in this way. The phage DNA was purified according to the method of Leder *et al.* (1977), digested by *Eco*RI and *Bam*HI and analysed by Southern blotting. Some of the restriction fragments were subcloned into pEMBL8 or pUC18 vectors (Yanisch-Perron *et al.*, 1985).

Preparation of total poly(A)-rich RNA

Cells were collected by centrifugation and washed three times with sterile phosphate-buffered saline (PBS). Total RNA was prepared by adding 20 ml of 3 M LiCl, 6 M urea to each gram of cells and the mixture was homogenized in a Teflon glass Potter homogenizer. The rest of the preparation was as described by Auffray and Rougeon (1980). Poly(A)-rich RNA was prepared from total RNA by chromatography on oligo(dT)-cellulose.

Protein synthesis in vitro

Proteins were synthesized in a reticulocyte lysate system according to Pelham and Jackson (1976). The standard incubation mixture, final volume 12 μl, con-

tained 5 μl of reticulocyte lysate, 0.5 mM dithiothreitol, 1 mM ATP, 200 M GTP, 20 mM creatine phosphate, 0.5 M CTP, 85 mM KCl, 1.2 mM magnesium acetate, 0.5 mM spermidine, a mixture of 19 unlabelled amino acids lacking methionine (50 M each), 6 μCi [^{35}S]methionine (Amersham) and ~0.25 μg of poly(A)-containing RNA of human cells. Incubation was performed at 30°C for 60 min.

Two-dimensional electrophoresis

The two-dimensional protein separation method was modified from the O'Farrell procedure (1975). The pH ranges of the ampholytes (Ampholines MKB) used in the isoelectric focusing gels were 5.8 and 3.5–10 (4/1). Treated samples were applied immediately to 13 cm tube gels and electrophoresis was carried out for a total of 8000 V × h. The second dimension was made on 25 cm SDS–acrylamide slab gels containing 8% acrylamide (Bio-Rad), 0.09% bisacrylamide (Bio-Rad), 0.1% SDS, Tris-HCl 0.375 M pH 8.8, 0.02% ammonium persulfate (Merck) and 0.06% Temed (Bio-Rad).

Electrophoresis was carried out for 15 h at 8 mA and for 2 h at 10 mA in a buffer containing 2.88% glycine, 0.6% Tris and 0.1% SDS. Gel staining was performed using 0.25% Coomassie Blue R 250 in a methanol/acetic acid/H_2O solution (5/1/5, by vol.). Industrex A Kodak films were used for autoradiography.

Cells (Table III)

Burkitt's lymphoma cells, JI, JBL2 and BL13 were established in the Centre International pour la Recherche contre le Cancer, Lyon (G.Lenoir).

B lymphocytes, fibroblasts, and SV-transformed fibroblasts were obtained from M.Fellous (Institut Pasteur). Lymphoid cells were grown in suspension with RPMI 1640 medium containing 10% fetal calf serum. Fibroblasts were grown in Dulbecco's modified minimal essential medium containing 10% calf serum.

DNA

High mol. wt DNA was prepared from cells as described by Blin and Stafford (1976). Restriction enzyme-generated DNA fragments were isolated from 1% agarose gels using DEAE–cellulose paper as described by the suppliers.

RNA blotting and hybridization

RNA electrophoresis under denaturing conditions in the presence of formamide, transfer to nitrocellulose and hybridization were carried out according to Goldberg (1980) using *Escherichia coli* DNA.

Hybridizations of 5 × 10^6 c.p.m./ml ^{32}P-labelled nick-translated probes to filter-bound RNA were performed at 42°C for 15 h. Following hybridizations, filters were washed first three times at room temperature with 2 × SSC, 0.1% SDS, for 15 min each, twice at 65°C wtih 1 × SSC, 0.1% SDS and then twice at 65°C for 30 min each.

DNA blotting and hybridization

DNA electrophoresis, transfer to nitrocellulose and hybridization were carried out according to Southern (1975) using *E. coli* DNA. Hybridization of 5 × 10^6 c.p.m./ml ^{32}P-labelled nick-translated probes to filter-bound DNA were performed at 42°C for 15 h. Following hybridizations, filters were washed under the same conditions as for the RNA hybridization.

Hybridization selection

Selection of specific RNA was performed essentially as described by Parnes *et al.* (1981) except that pre-hybridization was performed at 50°C for 4 h. 100 μg of total RNA was used for each experiment.

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